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The invention relates to a composition for killing the parasitic bee mite *Varroa Jacobsoni* containing as active ingredient one or more cyclic or acyclic terpenes, preferably linalool, linalyl acetate, eugenol and/or anethole or the volatile oil of plants containing them or their mixture.

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COMPOSITION FOR KILLING THE PARASITIC  
BEE MITE VARROA JACOBSONI

The invention relates to a composition for killing the parasitic bee mite Varroa jacobsoni.

5 Background of the invention

The Asian parasitic bee mite (Varroa jacobsoni) is a very serious problem in beekeeping and several methods and substances had been developed for controlling it.

Among such methods the biological methods of  
10 control make use of the knowledge of bee and Varroa biology and attack the mites where they are vulnerable. Such methods are: regularly removing the drone brood from the bee families, removing the brood at regular intervals and keeping it in incubators for the rest of time. These  
15 methods of mite control require however the highest skills, sophisticated equipment and both much effort and a highly concentrated working.

A physical method of mite control includes heat treatment of the colonies at temperatures of from 46 to  
20 48 °C. But this is a very risky and costly method as the bees may easily be killed if the temperature rises to 49 or 50 °C.

Chemical treatments are also known. These use chemical substances in dust or spray form which may  
25 easily have access to the final product honey, rendering such honey unfit for human consumption.

Substances which should be eaten by the bees are usually dissolved in sugar syrup and then sprayed onto

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bees; they are swallowed by the bees licking each other. One such substance is Perisine (Ritter, W.: Tierärztlicher Umschau, 40 /1/, 14-15, 1985) which has very favourable effects but can easily be passed over to the honey.

5 Therefore, Perisine cannot be used when the colony is collecting honey.

Another method of control is fumigation using substances like bromine propylate (Poltev, V.J. - Likhotin, A.K.: Veterinarija, 11, 60-61, 1975) or Amitraz (Kilani et  
10 al.: Apidologie, 12 /1/, 31-36, 1981). The use of these substances bears many risks as these chemicals are toxic, the risks are the following:

inhaling the gases when the fumigation stripes are being lit is highly dangerous to humans,

15 when using these chemicals obligatory wait periods are to be kept as determined by foodstuff legislation,

these chemicals cannot be used during the 14-day period prior to extracting the honey from the honeycomb,

these chemicals should be inserted or introduced  
20 preferably in the evening when all bees are again in the hive,

during summer bees get more aggressive after the application of the chemicals.

In some control methods chemicals like formic  
25 acid are evaporated. 10 ml of formic acid has to be evaporated daily, but it is difficult to controll the rate of evaporation. When the rate of the evaporation of formic acid is too high, the bees may be killed very easily.

The object of the present invention is to provide a  
30 composition which is effective in killing the mites but

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will not affect the life and health of the colony and will not affect the quality of honey produced.

Brief description of the invention

5 The invention is based on the recognition that mites can be effectively killed with compositions containing one or more acyclic or cyclic terpenes, preferably linalool, linalyl acetate, eugenol and/or anethole or the volatile oil of plants containing one or more acyclic or cyclic terpenes, preferably linalool, linalyl acetate, eucalyptol,  
10 eugenol and/or anethole or a mixture of these volatile oils or a mixture of these volatile oils and the terpenes mentioned above.

Detailed description of the invention

15 The compositions of the present invention can be prepared in forms suitable for evaporation, spraying or fumigation.

According to the invention the ready to use compositions for mite control usually contain from 0.001 to 1 % by weight of active ingredient, one or more usual  
20 carriers and if desired other known additives.

The preferred compositions contain as active ingredient anethole, linalool or linalyl acetate or volatile oil of plants containing them or their mixture.

As volatile oil of plants the following are  
25 preferred: oil of lavender, bergamot oil, oil of clove, anise oil. The main constituents of oil of lavender are linalool and linalyl acetate, while bergamot oil contains linalyl acetate as its major component. Oil of clove contains mainly eugenol, while the major component of  
30 anise oil is anethole.

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The advantage of the compositions of the present invention is that they contain natural substances as active ingredient, by the use of the composition bee mites can be effectively killed and they are harmless to bees and to the quality of honey produced.

The following tests show the activity of the compositions of the present invention.

#### Test 1

##### Laboratory evaporation test

10 In Petri-dishes of 10 cm diameter filter papers each of 2 cm<sup>2</sup> surface were placed and the substances to be tested were dropped thereon (20  $\mu$ l on each piece of paper). Then in each Petri-dish 3 bees each infested with one Varroa jacobsoni mite were placed and the behaviour of  
15 both bees and mites was observed.

The substances used and the results obtained are the following:

- |                  |  |
|------------------|--|
| linalool:        | the mite left the bee after 7 minutes,<br>the mite died after 12 minutes;    |
| 20 oil of clove: | the mite left the bee after 15 minutes,<br>the mite died after 18 minutes;   |
| anise oil:       | the mite left the bee after 5 minutes,<br>the mite died after 30 minutes;    |
| oil of lavender: | the mite left the bee after 5 minutes,<br>25 the mite died after 20 minutes; |
| linalyl acetate: | the mite left the bee after 7 minutes,<br>the mite died after 42 minutes;    |
| anethole:        | the mite left the bee after 6 minutes,<br>the mite died after 40 minutes;    |

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1 to 1 mixture (by volume) of oil of lavender and linalool:  
the mite left the bee after 9 minutes,  
the mite died after 10 minutes.

In all Petri-dishes the bees remained healthy.

5

Test 2

## Evaporation test in modular hives

Evaporation plates of 20 x 30 cm size were made from the mixture of one part of gypsum and two parts of diatomite. The plates were placed into the hives and 5-5  
10 ml of the substances to be tested were dropped on the plates. After 21 or 23 days the killed mites were counted and the killing effect was calculated. The results are summarized in Table 1.

Table 1

15	Active ingredient	Duration of treatment (days)	Mite killing effect (%)
	A	21	88
	B	21	93
	C	21	70
20	D	23	85

A: 1:1/v:v mixture of oil of lavender and linalool,  
B: 1:1/v:v mixture of anise oil and oil of lavender,  
C: oil of lavender,  
D: anise oil.

25 Remark: After each treatment the colony remained calm and healthy, and the brood was not deformed or otherwise affected, and adult bees hatched from this brood were healthy.

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Test 3

## Laboratory feeding test

1 % and 1% solutions of anethole, linalool and oil of lavender were prepared with 50 % by weight aqueous sugar syrup. 20-20 bees infested with mites were fed with these solutions for 15 days. It was observed that after 2 or 3 days mites started to die, all mites died within 15 days. The treatment had no harmful effect on the bees.

10

Test 4

## Spraying test in modular hives

1 % solutions of anethole, linalyl acetate and linalool were prepared with 50 % by weight aqueous sugar syrup and the solutions were sprayed on mite-infested bees being on the frames or into the spaces between honeycombs. 150 ml of the spray solution were applied to each three storied modular hive. On the day after treatment the dead mites and bees, if any, were counted and the activity was calculated.

20

The results thus obtained were then compared to the results obtained by using the commercially available "Varrescens" fumigation strips, the activity of the Varrescens strips was assumed to be 100 %. The results are shown in Table 2.



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Table 2

Active ingredient tested	Activity (%)
Anethole	129.86
Varrescens	100
5 Linalyl acetate	112.25
Varrescens	100
Linalool	133.13
Varrescens	100

Remark: The active ingredient of the Varrescens strip is  
10 Amitraz.

No bee was harmed during these treatments and no robbery activities could be observed.

Test 5

## Laboratory fumigation test

15 Mite-infested bees were placed in queen cages and the cages were placed in 5-litre glass containers. On a wringled piece of paper towel (size: 8 x 8 mm) 0.1 ml and 0.05 ml of the substances to be tested were dropped. The paper towel pieces thus treated were placed into a bee  
20 smoker and burned. From the fumes 10 or 12 blasts were introduced into the glass containers which were then covered with a glass plate. The glass plate cover had been removed after 10 minutes. The number of the dead mites (and bees) was counted after 24 hours. The results  
25 are shown in Table 3.

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Table 3

	Active ingredient	Amount of the active ingredient (ml)	<u>Number of</u>		Dying of the mites started after minutes	<u>Living</u>	
			bees	mites		bees	mites
			at the beginning of the test	at the beginning of the test		at the end of the test	at the end of the test
5	Anethole	0.1	14	10	5	12	0
		0.05	10	8	10	8	0
	Linalyl acetate	0.1	8	3	10	5	0
		0.05	9	9	10	9	0
	Linalool	0.1	12	9	10	11	0
10		0.05	11	3	10	10	0

Test 6

## Fumigation test in hives

Test 5 was repeated in hives, the substances to  
 15 be tested were used in an amount of 0.1 ml. At the time  
 of the test the outside temperature was 7 °C.

After one fumigation in case of anethole the number  
 of the dead mites was 9.7, this number was 3.0 for linalyl  
 acetate and 5.1 for linalool. No bee was killed in these  
 20 tests, the average activity was 90 %.

These tests were repeated by applying 0.1 ml of each  
 of the above active ingredients. The activity increased  
 to 99 % no bee was killed.

The compositions of the present invention are  
 25 illustrated by the following Examples:

Example AEvaporation plate

- a) On a 20 x 30 cm falt plate (thickness: 2 mm) 5 ml of  
 oil of lavender are applied:

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- b) On a 20 x 30 cm (thickness: 5 mm) plate made by mixing one part of gypsum with 2 parts of diatomite, 5 ml of a 1:1/v:v of anethole - ethanol mixture are applied.
- 5 c) On a 20 x 30 cm (thickness: 5 mm) plate made by mixing one part of gypsum with 2 parts of diatomite, 5 ml of a 1:1/v:v of linalool - paraffin oil mixture are applied.

Example BFumigation mixture

- 10 a) Fumigation strip  
A 3 x 10 cm paper strip is impregnated with a 10 % by weight aqueous potassium nitrate solution. On this paper strip 0.15 ml of anethole is applied.
- b) Fumigation pill or pellet
- 15 10 mg of linalool, 90 mg of cyclodextrin and 5 mg of potassium nitrate are mixed.

Example CSpraying mixture

- To 1 litre of aqueous sugar syrup containing 50 %  
20 sugar 2 ml of linalool and 1 ml of ethanol are added.

Example DSpray concentrate

- Two parts (by volume) of linalool are mixed with one part of ethanol. Prior to use this concentrate is diluted  
25 with an aqueous sugar syrup up to the desired concentration.

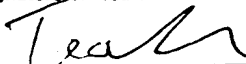
- 10 -

## Claims

1. Composition for killing the parasitic bee mite *Varroa jacobsoni* characterized by containing as active ingredient one or more acyclic or cyclic terpenes, preferably linalool, linalyl acetate, eugenol and/or anethole together with one or more carriers and/or other known additives.
2. Composition for killing the parasitic bee mite *Varroa jacobsoni* characterized by containing as active ingredient the volatile oil of plants containing one or more acyclic or cyclic terpenes, preferably linalool, linalyl acetate, eugenol and/or anethole or a mixture of these volatile oils together with one or more carriers and/or other known additives.
3. Composition for killing the parasitic bee mite *Varroa jacobsoni* characterized by containing as active ingredient a mixture of one or more acyclic or cyclic terpenes, preferably of linalool, linalyl acetate, eugenol and/or anethole and volatile oils of plants containing them together with one or more carriers and/or other known additives.
4. The composition according to Claim 1, characterized by containing as active ingredient anethole, linalool or linalyl acetate.
5. The composition according to Claim 2, characterized by containing as active ingredient anise oil or lavender oil.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/HU 89/00057

<b>I. CLASSIFICATION &amp; SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>1</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC <sup>5</sup> : A 01 N 27/00, 31/00, 31/02, 31/04, 31/06, 31/08, 31/14, 31/16, 49/00		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. <sup>5</sup>	A 01 N 27/00, 31/00, 49/00	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	GB, A, 2 163 651 (RIVIANA FOODS INC.), 05 March 1986 (05.03.86), see abstract.	(1-4)
X	GB, A, 2 178 315 (UNIVERSITY OF SOUTHAMPTON), 11 February 1987 (11.02.87), see abstract; claims.	(1-4)
X	US, A, 4 379 168 (DOTOLO), 05 April 1983 (05.04.83), see abstract.	(1-3)
X	Soviet Inventions Illustrated, Section Ch, week J51, issued 1983, February 9 by Derwent Publication Ltd. (London), see SU-908-296 (ASIP).	(1-3)
X	US, A, 4 845 131 (BEHRENZ et al.), 04 July 1989 (04.07.89), see abstract.	(1-3)
A	DD, A1, 232 181 (VEB CHEMIEKOMBINAT BITTERFELD), 22 January 1986 (22.01.86), see abstract.	(1)
A	DD, A1, 255 875 (VEB FETTCHEMIE), 20 April 1988 (20.04.88), see abstract.	(1)
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>14</sup> Special categories of cited documents: <sup>15</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"G" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
05 July 1990 (05.07.90)	12 July 1990 (12.07.90)	
International Searching Authority	Signature of Authorized Officer	
AUSTRIAN PATENT OFFICE		

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Chemical Abstracts, Volume 107, no. 25, issued 1987, December 21 (Columbus, Ohio, USA), W. Stoya et al. "Lactic acid for the treatment of varroato-sis and its effect on honey" see page 647, column 2, the abstract no. 235 042g & Dtsch. Lebensm.-Rundsch. 1987, 83(9), 283-6.	(1)
A	DE, A1, 3 538 688 (BAYER AG), 07 May 1987 (07.05.87), see claims.	(1)
A	Chemical Abstracts, Volume 107, no. 11, issued 1987, September 14 (Columbus, Ohio, USA), D. Popeskovic et al. "The blockade of hemocyanin function of the mite Varroa jacobsoni as a specific physiological basis for systemic treatment of honeybee varroatosis" see page 27, column 2, the abstract no. 89 361s & C.R. Seances Soc. Biol. Ses. Fil. 1986, 180(6), 663-8.	(1)
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Anhang zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

Annex to the International Search Report on International Patent Application No. PCT/HU 89/00057

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned International search report. The Austrian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Annexe au rapport de recherche internationale relatif à la demande de brevet international n°.

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Patent document cited in search report  
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Datum der Veröffentlichung  
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Patent family member(s)  
Membre(s) de la famille de brevets

Datum der Veröffentlichung  
Publication date  
Date de publication

GB-A - 2163651

AU-A1-45357/85 20-02-86  
BE-A1- 902977 27-01-86  
DE-A1- 3526911 13-03-86  
FR-A1- 2571220 11-04-86  
GB-A0- 8518903 04-09-85  
GB-A1- 2163651 05-03-86  
JP-A2-61083103 26-04-86  
NL-A- 8502137 17-02-86  
PT-A- 80862 01-08-85  
PT-B- 80862 09-01-87  
US-A- 4933371 12-06-90

GB-A - 2178315

BR-A - 8605335 31-05-88  
GB-A0- 8519540 11-09-85  
GB-A1- 2178315 11-02-87  
US-A - 4820513 11-04-89

US-A - 4379168

05-04-83

US-B1- 4379168 23-01-90  
US-B1- 4379168 23-01-90  
US-B1- 4379168 23-01-90

US-A - 4845131

04-07-89

AU-A1-14373/88 13-10-88  
DE-A1- 3711910 20-10-88  
EP-A2- 285983 12-10-88

DD-A1- 232181

22-01-86

None

DD-A1- 255875

20-04-88

None

DE-A1- 3538688

07-05-87

AT-E -	52407	15-05-90
BR-A -	8605350	04-08-87
DE-C0-	3670949	13-06-90
DK-A0-	5198/86	30-10-86
DK-A -	5198/86	01-05-87
EP-A1-	224697	10-06-87
EP-B1-	224697	09-05-90
HU-A2-	44421	28-03-88
HU-B -	199251	28-02-90
JP-A2-	62108803	20-05-87

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